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# Sulfur and Methionine Supplementation with Urea for Feedlot Cattle

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# SULFUR AND METHIONINE SUPPLEMENTATION WITH UREA FOR FEEDLOT CATTLE

BY

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SULFUR AND METHIONINE SUPPLEMENTATION

WITH UREA FOR FEEDLOT CATTLE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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# INTRODUCTION

Protein is often a limiting nutrient in animal diets. As ruminant animals have the ability to convert large quantities of nonprotein nitrogen to protein, the cost of protein supplementation can be greatly reduced by substituting cheaper nonprotein nitrogen compounds in protein supplements for certain amounts of preformed protein. This process is mediated through rumen microorganisms and, to date, urea has been the most widely used nonprotein nitrogen compound in ruminant diets.

Efficiency of urea utilization may be affected by several factors. Among these are level and source of energy, level and source of protein and percent urea in the total diet and in the supplement. Other essential nutrients in the diet should also be properly balanced. There has been a tendency in recent years to use higher levels of urea than was earlier recommended. Under some conditions, urea has been satisfactory as the only source of supplemental protein to diets for cattle. However, consideration must be given to likely problems of palatability and potential toxicity as well as efficiency of urea utilization.

Although lower feed costs have resulted from substituting cheaper nonprotein nitrogen sources for preformed protein, animal performance has often been at a lower rate. This has prompted investigations towards improvement in utilization of urea nitrogen by use of various supplements with urea. There has been a considerable amount of interest in possible effects from amino acid supplementation since protein needed by animals is a need for amino acids. Some experiments have shown that the sulfur-containing amino acids are the first limiting ones when ruminants are fed diets with urea furnishing practically the only source of nitrogen. It has also been shown that rumen microorganisms are able to synthesize the sulfur-containing amino acids when ample sulfur is present. More information is needed as to the possible benefits of amino acids, particularly the sulfur-containing ones, and sulfur supplementation when urea is used as the only supplemental protein under various conditions of protein and energy levels in the basic diets.

The objective of this research was to study effects of sulfur and methionine hydroxy analogue additions to high-urea supplements fed to growing-finishing beef cattle. A feeding trial was conducted in which weight gains, feed consumption and feed efficiency were used as measures of performance.

# REVIEW OF LITERATURE

Extensive reviews of the reported research on utilization of urea and other nonprotein nitrogen compounds have been published by several workers (Reid, 1953; Annison and Lewis, 1959; Hungate, 1966; Chalupa, 1968; Loosli and McDonald, 1968; Helmer and Bartley, 1971). Stangel, Johnson and Spellman (1963) also presented an extensive compilation of abstracts and titles of research papers dealing with the use of urea and other nonprotein nitrogen compounds in ruminant diets.

Research reviewed in these several publications has shown that when urea from feed sources enters the rumen it is rapidly dissolved and hydrolyzed to ammonia and carbon dioxide by bacterial urease. Experiments dealing with nitrogen balance, ruminal ingesta composition and isotopic tracers have been employed to demonstrate that urea nitrogen is converted to and stored as protein nitrogen. Subsequent enzymatic digestion of the microorganisms avails the host animal of the protein contained therein.

Efficient utilization of the nitrogen from urea depends on the ability of microorganisms in the rumen to convert ammonia to bacterial protein. Amino groups may be split from amino acids and from intact proteins and used by bacteria in the same manner. Ammonia is readily absorbed from the rumen and thus is available to the microbes for only a limited period of time. Factors which affect either the rate of release of ammonia from urea or the rate of assimilation of microbial protein from ammonia will therefore affect the efficiency of urea utilization.

Levels and sources of energy and protein markedly affect urea utilization. A readily available source of energy has been shown to be necessary for efficient utilization of urea. Some true protein in the diet may be necessary for urea utilization. Urea has been shown to be utilized rather efficiently in diets with up to 12% protein and less efficiently in diets containing higher levels of protein. However, diets for cattle and sheep seldom need to exceed 12% protein, and this limitation as to level of protein and efficiency of nitrogen utilization would be applicable to sources of preformed protein as well as to urea.

A major problem in efficient utilization of urea is the rapid release of ammonia. Limited work has been directed towards improving urea utilization by reducing the rate of urea hydrolysis by inhibiting rumen urease, but none of several methods investigated has proven entirely satisfactory. A number of attempts have been made to slow ammonia release from urea by coating it with water insoluble materials thereby reducing its solubility and enabling greater quantities to be used, but this approach has met with limited success also. Another means of minimizing ammonia nitrogen losses is by using nonprotein nitrogen compounds less readily hydrolyzed in the rumen than urea. To date, of all the nonprotein nitrogen compounds considered as substitutes for urea, biuret probably has received the most attention as it is more slowly hydrolyzed by rumen microorganisms and is less toxic than urea to ruminants.

Generally accepted guidelines for limitations in use of urea in the past have been not more than 3% urea in the concentrate mix, less than 1% urea in the diet dry matter or not more than one-third to one-half of the protein equivalent of the total diet. Consideration must be given to likely problems of palatability and potential toxicity as well as efficiency of urea utilization. Therefore, each of these limitations may be important under some conditions. However, diets for finishing cattle and sheep generally contain high levels of energy and frequently are only mildly deficient in protein. Urea as the only supplemental source of protein in such diets is not likely to present any problems because of the small quantity included.

Factors affecting utilization of urea and other nonprotein compounds become more important with decreasing levels of dietary energy and higher levels of urea supplementation. At least two areas of concern would appear to be the possible benefits from amino acid supplementation and the need for added sulfur. Additions of amino acids and sulfur have increased performance in some cases but were without effect in others. A few experiments have been conducted in the past on these subjects; however, much work has been done in recent years to determine the benefits from supplements of amino acids and sulfur, particularly as they are used in conjunction with high-urea diets. It was the objective of the research conducted by the author to study benefits from methionine and sulfur supplementation with urea. Therefore the literature review will be directed more specifically to these and related areas.

<u>Effect of Protein on Urea Utilization</u>

Urea utilization has been shown to be affected by the protein content of the diet. This effect appears to be influenced by solubility of the protein as well as by the amount.

*In vitro* experiments (Wegner *et al.*, 1940) showed that the level of protein in a medium inoculated with rumen microorganisms influenced the rate of conversion as well as the amount of urea converted to protein. As the concentration of casein in the artificial rumen was increased, the amount of urea converted to protein decreased. The effect of protein level on urea utilization was also studied by Wegner *et al.* (1941) by feeding a fistulated heifer twice daily a diet consisting of 15 lb. corn silage, 4 lb. timothy hay and 4 lb. of a corn-oat mixture. Linseed meal or urea was varied to increase the protein level of the concentrate portion of the diet from 11.3 to 31.1%. Samples of the rumen contents were collected and analyzed for nonprotein nitrogen, total nitrogen and dry matter. Although it was found that some urea was converted to protein when the crude protein content of the concentrate mixture supplemented with linseed meal was 20 to 24%, the utilization of urea nitrogen decreased substantially at protein levels above 18%. When the diet was not supplemented with linseed meal, increasing additions of urea to a level of 4.5% (a protein equivalent of approximately 12% in the diet) were utilized efficiently. This combination of urea, corn and oats provided a concentrate mixture containing approximately 23% in protein equivalent.

Belasco (1954) suggested that the availability of nitrogen from all sources was important for good cellulose digestion, presumably through growth promotion of cellulose digesting bacteria. When urea was fed in a 1:1 mixture with feed proteins, utilization *in vitro* was very efficient and the activity of the mixture was better than with feed protein alone. An increase in cellulose digestion as well as in the amount of urea utilized resulted when the level of urea as the sole source of nitrogen was increased.

Adding urea to a basal diet in amounts to produce the equivalent of 12% crude protein on a dry basis induced a retention of nitrogen in growing lambs that could not be improved by raising the true protein content of the diet (Johnson *et al.*, 1942). Hamilton *et al.* (1948) compared the utilization of nitrogen of urea with that of some feed proteins (dried skim milk, dried skim milk plus cystine, gluten feed, casein or casein plus cystine and linseed meal). Urea was shown to be as satisfactory a source of nitrogen for growing lambs as that from most ordinary feeds providing at least 25% of the total nitrogen was in the form of preformed protein and provided further that the total protein equivalent of the diet did not exceed about 12%.

Urea in amounts to provide one-half or all the supplemental nitrogen in diets with 6, 9 or 12% protein was compared with cottonseed meal in a wintering diet for steer calves fed a low-quality native meadow hay (Raleigh and Wallace, 1963). Level of nitrogen significantly affected the digestibility of cellulose, dry matter and organic matter with 9 or 12% crude protein levels having higher values than the 6% level or hay alone (5.5% crude protein). Nitrogen digestibility significantly increased with each increase in nitrogen level of the diet regardless of the source of supplement. There was, however, an interaction of source and level of nitrogen in the 6 and 9% protein diets on nitrogen digestibility. Nitrogen digestibility was lowest at the 6% level but highest at the 9% level when urea was the supplemental nitrogen.

Data were presented by Preston _et al._ (1964) which showed that gain and feed efficiency are affected by protein to energy (P:E) ratio. In an experiment involving eight lambs, various ratios of crude protein (mg) to energy (kcal of estimated net energy) were fed in an extra period Latin Square change-over design to determine the effects of these ratios upon blood urea nitrogen, gain and feed consumption. Each period was 21 days during which the lambs were full-fed a diet of 50% cottonseed hulls, 8% cane molasses, 0.9% minerals and vitamins and a variable percent of corn and soybean meal to give P:E ratios of 54, 70, 102 and 118 mg protein per kcal ENE. Daily gains increased ($P<.005$) with increasing P:E ratio (61, 168, 251 and 251 g). Daily feed consumption also increased ($P<.05$) with increasing P:E ratio (1700, 1800, 1920 and 1890 g). Blood urea nitrogen increased ($P<.005$) with increasing P:E ratio (2.5, 3.5, 10.0 and 14.5 mg per 100 ml). These data indicate that gain would be maximal and feed per unit of gain minimal at a P:E ratio of 83 and 73, respectively.

Results of the research reviewed showed that efficiency of urea utilization is influenced by level of protein in the diet and rate in which it becomes available. Urea appeared to be a satisfactory source of nitrogen up to at least 50% of the total dietary nitrogen and when the total protein equivalent of the diet does not exceed about 12%. A protein level of 12% is generally considered adequate under most conditions for cattle. Therefore, this limitation on protein level would not be peculiar to urea. The protein to energy ratio has been shown to influence the efficiency of protein utilization. Safety and efficiency in utilization of urea, therefore, improves with increasing levels of energy in relation to the nitrogen.

## Amino Acid Supplementation

Ruminants reduce the biological value of high-quality proteins and increase the value of low-quality proteins as determined for non-ruminants making feed protein characteristic in biological value to that of mixed microbial protein. Bergen *et al*. (1968) studied the effect of different high-energy, isocaloric diets on the bulk amino acid composition, protein quality and *in vitro* digestibility by rumen bacteria, rumen protozoa and total rumen microbial cell mass. Their results showed that the bulk amino acid composition and protein quality were not affected by a number of various changes in diets. This substantiated work by Duncan *et al*. (1953) who found that amino acid composition of ruminal fluid proteins was similar whether animals were fed natural diets with preformed protein or purified diets with urea as the source of nitrogen. Meyer *et al*. (1967) made the same conclusions when the diets compared were a succulent roughage (alfalfa pasture), a dried roughage (alfalfa hay) or grain sorghum-alfalfa hay. However, Little et al. (1965) found ruminal protein to be of higher biological value from concentrate-fed steers than from hay-fed steers.

Amino acids have been employed in nitrogen balance studies in which they have been administered both orally and by abomasal infusion by-passing the rumen. The supplementation of lysine has been of particular concern to researchers because of its low content in corn and its low solubility in the rumen. Devlin and Woods (1964) failed to increase nitrogen retention by steers fed diets with corn and corn gluten meal when 9 or 18 g of lysine were fed per steer daily. However, there was significantly more nitrogen retention in the control steers and in those steers infused with 9 g of lysine into the abomasum than for steers fed lysine.

Hale et al. (1959) reported that 900 g of L-lysine hydrochloride added per ton of a complete pelleted diet resulted in lamb gains of 0.42 lb. per day compared to 0.32 lb. for control lambs fed a diet containing 53.5% ground corn, 35% alfalfa meal, 7% molasses and 1% urea in a 68-day trial. On the other hand, Harbers et al. (1961) added 990 g of L-lysine hydrochloride per ton to a 11.8% protein, high-energy diet composed mainly of milo and cottonseed meal and observed no advantage from the lysine supplementation. Other workers (Oltjen et al., 1960; Meacham et al., 1961) have reported no advantage from feeding lysine to sheep receiving purified diets.

Researchers at Purdue conducted a series of trials with oral administration of amino acids to cattle. In the first trial (Gossett et al., 1962), they found that adding L-lysine at 10 g per head daily to supplements containing different levels of urea fed with ground ear corn consistently improved daily gains from 5 to 13% and feed efficiency from 1 to 4%. The average increase in gain (2.24 vs. 2.09 lb. per day) between the six pens receiving lysine and the controls was highly significant. The addition of lysine to a supplement containing only natural protein was of no benefit, but adding it to urea-containing supplements made them equivalent to the all-natural protein supplement when fed on a protein equivalent basis. In the second trial conducted under similar conditions (Gossett et al., 1962), 10 g of lysine per head daily again increased rate of gain making the performance of cattle fed a high-urea supplement nearly equal to that obtained from all-natural protein diets. However, in the third trial (Gossett et al., 1962), the addition of lysine to a high-urea supplement at either the 5- or 10-g level resulted in a reduction in daily gains, but feed conversion was improved with the 10-g level of the amino acid.

Kolari et al. (1961) conducted two experiments in which four levels of protein from linseed meal plus limited ground ear corn and a full feed of corn silage were fed, with and without lysine, to 800-lb. heavy yearling steers and to 480-lb. heifers. They were unable to obtain an advantage from 10 g of lysine per head daily under these conditions.

Methionine at 10 g per head daily and a combination of lysine and methionine each at the 10-g level were also included as experimental treatments in the second trial of Purdue workers (Gossett et al., 1962). Methionine improved the high-urea supplement slightly, but not to the extent of lysine. The combination of lysine and methionine was of no benefit when added to either an all-natural protein or a high-urea supplement.

Methionine supplementation has generally been of more concern to researchers than lysine as methionine is considered the first limiting amino acid in many diets for ruminants. Klosterman et al. (1951) reported that the addition of DL-methionine significantly increased nitrogen retention and exhibited greater effects with certain diets than with others when fed to sheep. Methionine at 3 or 6 g per head daily increased nitrogen retention and digestion when added to a 10.2% protein diet containing urea, but the additions failed to improve results when used with the same diet (7.2% protein) without urea. The methionine hydroxy analogue significantly improved retention of nitrogen and digestion of crude fiber and dry matter when fed in high-urea diets to lactating dairy cows (Polan et al., 1970).

Barth et al. (1959) found that supplementation of a high-urea diet (87% of total dietary nitrogen) with either methionine or tryptophan or both amino acids increased ($P < .05$) the percentage retention of absorbed nitrogen. Digestibility of nutrients was not influenced by either amino acid supplementation.

Noble et al. (1955) fed practical diets of shelled corn, hay and beet pulp in three feedlot trials in which urea or soybean meal alone or in combination with 2 g of methionine per head daily were compared to a low-protein basal diet for fattening lambs. The addition of methionine to the urea diet resulted in small but consistent increases in weight gains and feed efficiency, which in two of the three trials were nearly equal to the soybean meal basal; however, its addition to the soybean meal diet was without effect.

Burroughs and Trenkle (1969) added levels of 0, 0.3, 0.6, 1.2 or 2.4 g of methionine hydroxy analogue to natural diets, either with or without urea, and fed to lambs in a short 35-day trial. They reported a favorable response from methionine analogue in these isonitrogenous diets both with and without urea. The lower levels gave more favorable responses. Daily gains from the above methionine levels were, respectively, 0.40, 0.47, 0.45, 0.41 and 0.39 lb. per day for lambs receiving urea and 0.39, 0.42, 0.49, 0.42 and 0.32 lb. for those not receiving urea. Corresponding feed requirements per hundredweight of gain were, respectively, 690, 602, 613, 576 and 718 lb. for the urea fed groups and 710, 636, 518, 664 and 812 lb. for the nonurea-fed groups.

Gossett et al. (1962) fed 5 or 10 g of methionine hydroxy analogue daily to steers for 207 days. Steers received a high-urea supplement with ground shelled corn and corn silage. The 5-g level was of no benefit in daily gain, feed efficiency or carcass values.

The 10-g level gave a significant depression in rate of gain (2.43 vs. 2.13 lb.) and reduced daily feed intake (20.8 vs. 19.1 lb.).

In a later study, Beeson *et al.* (1970) found cattle fed 3 g methionine hydroxy analogue per head daily with an ear corn diet gained 7% less rapidly than controls (2.80 vs. 3.01 lb.). Feed intake was similar for the two groups (31.6 vs. 32.0 lb.) resulting in decreased feed efficiency for cattle receiving methionine analogue. In a study by Hale *et al.* (1970), methionine hydroxy analogue was not effective in increasing rate of gain or reducing feed requirements for steers when added to a milo diet containing 90% concentrates, 5% cottonseed hulls and 5% alfalfa hay. The beginning diet contained 0.70% urea and the finishing diet 0.50% urea. Daily rates of gain were 2.81 vs. 2.93 lb. and feed requirements per hundredweight of gain were 759 vs. 739 lb. for treated and control animals, respectively. Cattle were fed 22 g of methionine analogue daily for the first 56 days which reduced feed intake by 1 lb. daily and greatly reduced average daily gain (3.01 vs. 3.35 lb.). The methionine analogue was reduced to 0.5 g per lb. of diet at 56 days which supplied 11 g per animal daily for the last 84 days of the trial. Average daily gains were similar for this period (2.65 vs. 2.67 lb.).

In contrast to the foregoing results, Burroughs *et al.* (1969) found a marked response with heifers fed 3 g methionine hydroxy analogue per head daily with a diet of rolled corn and cobs supplemented with urea. Heifers were started on experiment at 460 lb. and terminated at approximately 810 lb. Methionine analogue-supplemented pens gained 13% faster (2.44 vs. 2.16 lb. daily) and required 10% less feed per hundredweight of gain (607 vs. 676 lb.). In a second trial, methionine hydroxy analogue additions to a urea supplement improved performance of steers in the first 72 days of the study only (Burroughs et al., 1970). Steers with initial weights of 745 lb. and final weights of about 1175 lb. were fed rolled corn and corn cobs for 133 days. During the first 72 days, adding 3 g of methionine analogue daily improved both rate of gain and feed efficiency by 7%. Adding 4.5 g daily gave 6% less response in rate of gain and 3% higher feed requirement. Negative results were obtained when 9 g of methionine analogue was added. No favorable response was obtained with any level of methionine analogue during the last half of the experiment. Adding 1.5 or 3 g of methionine analogue daily to all-vegetable proteins gave no response.

Lysine and methionine have been the amino acids most commonly supplemented to the diets of cattle and sheep, especially under conditions of high-urea supplementation. These amino acids have been shown to be of greatest limitation with diets composed largely of corn and when urea supplies the major source of supplemental protein. Research has shown some improvement from the supplementation of lysine, but generally results have been small and inconsistent. More emphasis has been directed towards methionine supplementation because it appears the more limiting than lysine in diets for ruminants and the fact that it contains sulfur. Feed composition data indicate an apparent close relationship between sulfur and protein contents. Conditions most suited for amino acid supplementation would appear to be when diets are low in protein and when urea or other source of nonprotein nitrogen is used for the needed supplemental protein.

Influence of Sulfur

Some of the beneficial effects reported from feeding methionine to ruminants on high-urea diets may represent a response from sulfur contained in the methionine. Rumen microorganisms synthesize sulfur-containing amino acids provided sulfur is present. Either inorganic or organic sulfur can serve for this purpose. Sulfur content of feeds appears to be closely related to the protein content. Sulfur analysis on feeds is rather meager and is given for only 33 feeds in a recent compilation on chemical composition (NRC, 1969). The average nitrogen to sulfur ratio for these feeds is 13.2 to 1. This level of sulfur to nitrogen is about that considered necessary to meet sulfur requirements for ruminant animals. Substituting urea for preformed protein in the diet removes a major source of sulfur. A low ratio of sulfur to nitrogen is likely to be encountered resulting in a deficiency of the element.

Thomas et al. (1951) demonstrated that a deficiency of sulfur limited nonprotein nitrogen utilization by sheep fed purified diets. The deficiency could be corrected by adding sulfate as the sole source of sulfur. Loosli (1952) suggested that tissue analyses gave an approximation of the sulfur requirement of sheep. Based on tissue analyses, he theorized that one part of sulfur was needed for each 15 parts nitrogen in the diet. Rozgoni (1961) obtained results that indicated 8.2:1 was the best nitrogen to sulfur ratio in a diet, even when tissue analyses were 15:1. This is in agreement with Moir et al.

(1967) who found that narrowing the nitrogen to sulfur ratio from 12:1 to 9.5:1 resulted in a corresponding improvement in nitrogen retention from 28.8 to 36.0%.

Starks et al. (1953) used paired feeding techniques to show that elemental sulfur could be used by sheep to supply the dietary need of sulfur when added to a low-protein diet where the major source of nitrogen was urea. Lambs receiving elemental sulfur retained more nitrogen ($P=.015$) and more sulfur ($P<.01$), their wool growth was increased ($P<.01$) and they came closer to maintaining their weight than those on the basal diet ($P=.033$). Starks et al. (1954) further studied the utilization of inorganic sulfur and urea in the nutrition of lambs in an experiment in which 40 growing-fattening lambs were fed a partially purified diet (consisting primarily of wood cellulose, wheat straw, starch, cerelose and wood). The basal diet, containing 0.054% sulfur with 92% of the nitrogen from urea, was supplemented with three levels of elemental sulfur (0.2, 0.4 and 0.6%), sodium sulfate (0.89, 1.33 and 1.78%) or DL-methionine (0.2, 0.5 and 0.7%). Weight gains and wool growth were increased by the addition of sulfur supplements and the increases were highly significant ($P<.001$). There was no statistical difference among the sulfur sources or levels within each source. However, Lofgreen et al. (1953) reported that the addition of 0.2% sodium sulfate to a basal diet made up of approximately 87% natural feeds, with urea furnishing 40% of the total nitrogen, was without effect on gains in body weight, efficiency of feed utilization, nitrogen retention, serum sulfate levels or wool growth.

Many researchers (Jones et al., 1952; Thompson et al., 1952; Davis et al., 1954; Gossett et al., 1962) were unable to show that animal performance, in terms of milk production or weight gains, was affected by adding supplemental sulfur to practical diets without urea. This lack of response to sulfur is attributed to most commonly used feedstuffs being adequate in sulfur content (Davis et al., 1954; Coombe et al., 1960). However some researchers have indicated that sulfur supplementation may be of some benefit in urea-containing diets. An increase of approximately 10% in body weight gains of heifers in an experiment conducted by Jones and Haag (1946) suggested that sodium sulfate may improve the utilization of urea in certain diets. One percent of sodium sulfate was added to the concentrate portion to provide 0.13% of sulfur in the diet. The basal concentrate mixture to which 3% of urea was added contained 6.2 to 7.6% crude protein. Brown et al. (1960) fed dairy heifers to determine the effect of level and source of sulfur on urea utilization. All heifers were fed 1 lb. corn cobs and 1.5 lb. corn silage per 100 lb. of body weight daily. Groups 1, 2, 3, 4 and 5 received a high-urea supplement, whereas group 6 received soybean meal. Groups 2, 3 and 4 were supplemented with sodium sulfate and group 5 with methionine hydroxy analogue. Nitrogen to sulfur ratios for groups 1, 2, 3, 4, 5 and 6, respectively, were 22.3, 6.4, 12.0, 16.4, 12.8 and 13.3. In the same order, the average body weight gains were

72.4, 94.3, 84.6, 97.7, 87.8 and 102.5 lb. All levels and sources of sulfur significantly increased daily gains ($P<.05$); however, the group receiving the conventional protein supplement made somewhat greater gains than those receiving urea. Only slight differences in feed consumption were observed among groups.

Nitrogen balance studies (Lofgreen et al., 1947; McLaren et al., 1959) showed that adding methionine to a diet containing urea increased retention of absorbed nitrogen by lambs. Goodrich et al. (1967) found that both sodium sulfate and elemental sulfur improved nitrogen retention of lambs fed urea.

Methionine, elemental sulfur and sodium sulfate are the principle compounds that have been studied as sulfur sources for ruminant animals. However, little information is available on the availability of these sulfur sources. Albert et al. (1956) reported that on the basis of total sulfur in the diet, only 30% as much sulfur was needed when fed as methionine as compared to elemental sulfur. The sulfur requirement of lambs was also met by feeding one-half as much sulfur from methionine as from sulfate. Johnson et al. (1970, 1971) used plasma levels and retention data to determine the value of $^{35}S$ from elemental sulfur, sodium sulfate and methionine. Retention data showed that sulfur from elemental sulfur and sodium sulfate were retained 38 and 80% as well as sulfur from methionine. These data suggest that methionine sulfur is utilized more effectively than elemental sulfur or sodium sulfate and that sodium sulfate is utilized more efficiently than elemental sulfur.

Various interrelationships exist between sulfur and other minerals. Dick (1956) was able to develop characteristic lesions of copper deficiency in the wool of sheep within 7 days after administering high doses of molybdenum and sulfate. This occurred despite the fact that liver copper was not depleted and plasma copper levels were elevated above normal. Nutritional muscular dystrophy, a myopathy condition encountered in young calves and lambs, has been reported to be influenced by several factors including the presence of sulfate. Muth _et al_. (1961) reported that the addition of 0.053% sulfur as sodium sulfate to the diet of ewes decreased the effectiveness of 0.1 ppm of added selenium in preventing nutritional muscular dystrophy in lambs. Goodrich and Tillman (1966) found that liver iron increased in sheep as the sulfur level of the diet increased. They interpreted this increase in liver iron to be a reflection of the influence that sulfate had on copper.

Some benefits have been demonstrated in ruminants from adding either inorganic or organic sulfur to purified diets low in sulfur and having a part or all of the supplemental protein replaced by urea. On the other hand, most investigations have failed to show a consistent advantage from adding sulfur to practical diets without urea or where urea made up a small percentage of the total nitrogen. However, diets low in preformed protein and using urea to furnish the major source of the total protein are generally low in sulfur content. Adding urea to these diets widens the nitrogen to sulfur ratio and sulfur supplementation had frequently been shown to be beneficial.

Quality of Protein Synthesized from Urea

Since Hart et al. (1939) found that a diet containing yellow corn, starch, timothy hay and urea, providing a protein equivalent of 9.44%, supported fairly good growth of calves, it was indicated that protein formed from urea was of at least fair quality. Harris and Mitchell (1941b) showed that diets too low in protein to support growth of lambs would promote appreciable growth when urea was added; however, growth response was not as great as when casein was fed.

Harris et al. (1943), in an experiment with calves and growing steers, showed that the biological value of urea nitrogen, fed at levels providing 80 to 90% of the total nitrogen of the diet, was 34 as compared to 60 for soybean meal nitrogen. In other experiments where urea nitrogen composed approximately 80% of the total dietary nitrogen, biological values of 45 to 50 were obtained (Johnson et al., 1944; Hamilton et al., 1948). It was concluded that urea fed at very high levels was utilized less efficiently than at lower levels. When nitrogen was provided by urea or casein at levels just maintaining equilibrium in lambs, biological values were 62 and 79, respectively (Harris and Mitchell, 1941a). The biological value of nitrogen of a corn silage diet supplemented with sufficient urea to provide 8, 11, and 15% protein equivalent were 74, 60 and 44, respectively.

Miller and Morrison (1942) found no difference in quality of protein provided by timothy, alfalfa and mixed timothy-alfalfa hays.

Lambs utilized nitrogen of soybean meal, linseed meal, corn gluten meal, casein and nonfat dry milk solids equally efficient. The nitrogen of diets in which urea composed more than one-half of the total nitrogen was used less efficiently than similar diets containing linseed meal or mixtures of linseed meal and smaller proportions of urea.

With increasing levels of dietary nonprotein nitrogen, the relative contribution of the bacterial and protozoal proteins becomes more important. Some insight as to the quality of microbial protein has been gained by amino acid analyses of bacterial and protozoal hydrolysates and by feeding microbial preparations to laboratory animals for nutritive value assessment. The constancy of the amino acid composition of rumen bacteria was demonstrated by Weller (1957), who examined bacterial preparations obtained from animals fed four different diets of wheaten hay chaff (0.89% nitrogen), lucerne hay chaff (2.9% nitrogen), mixed green pasture and wheaten straw, crushed oat grain and urea; and by Purser and Buechler (1966), who performed amino acid analysis on 22 strains of rumen bacteria grown in pure culture. However, Bergen _et al_. (1967) found considerable variation among individual strains studying protein quality of rumen bacteria using an _in vitro_ enzymatic digestion system. Furthermore, the proportion of essential amino acids released during the digestion of different bacterial strains also varied markedly, which suggests that modification of the bacterial population may be an important factor in the nitrogen status of an animal and its response to dietary changes.

Duncan et al. (1953) presented quantitative evidence that rumen microorganisms can utilize urea nitrogen to synthesize amino acids. They found that except for histidine, the amino acid pattern of the protein in the rumen contents of animals fed a urea-containing purified diet was fundamentally similar to that of animals fed a natural diet. However, Little et al. (1966) determined the free plasma amino acid concentrations of steers fed diets supplemented with either soybean meal or urea and found that substituting urea for soybean meal decreased plasma levels of lysine, iso-leucine, valine, proline and methionine.

Estimated biological values from nitrogen balance studies (Gallup et al., 1954) with steers receiving either urea or soybean meal indicated that soybean meal nitrogen was more efficiently utilized. Neither Embry et al. (1957) nor Freitag et al. (1968) were able to show a significant decrease in nitrogen balance attributable to urea.

Most studies have shown urea inferior to protein nitrogen sources, indicating that less absorbed nitrogen is retained for utilization by animal tissues. Reported biological value, plasma amino acid and microbial amino acid data suggest that lowered nitrogen retention may result from a deficiency in the kind and amount of amino acids synthesized by rumen microorganisms. Despite urea nitrogen not appearing capable of completely satisfying nitrogen requirements of rumen microorganisms and animal tissues, its value as a protein replacement is supported by many reports.

General Considerations

Better utilization of urea occurs when adequate energy levels exist in relation to the total protein of the diet. Further enhancement of urea utilization is possible when some preformed protein is fed, as in the case when diets contain natural feedstuffs. A source of added sulfur at a rate of 1 part sulfur to each 10 parts nitrogen from urea or other nonprotein nitrogen source appears to be a sound practice when these nitrogen substances make up a large portion of the total nitrogen in the diet. Beneficial effects from amino acid supplementation to diets adequate in total nitrogen and sulfur have not been well established.

# METHOD OF PROCEDURES

The experiment reported herein was initiated in February, 1971 and was conducted over a period of 224 days. It was designed (1) to compare various high-urea protein supplements to an all-vegetable protein supplement, (2) to determine the value of added sulfur in diets with urea as the only supplemental protein and (3) to determine the value of various sources of sulfur and/or methionine hydroxy analogue in diets for growing and finishing beef cattle with urea providing a major portion of the dietary nitrogen.

Ninety-six Hereford steer calves averaging about 500 lb. were used in this experiment. Prior to the start of the trial, the calves received a full feed of alfalfa-bromegrass haylage for approximately 3 weeks. The cattle were weighed for an initial filled weight and allotted at random after stratifying on basis of this weight. Initial shrunk weights were taken after with-holding feed and water for about 18 hours. Steers were then sorted into 12 pens of 8 animals each for 6 replicated treatments as follows:

1. soybean meal control
2. urea control
3. urea + sodium sulfate ($Na_2SO_4$)
4. urea + calcium sulfate ($CaSO_4 \cdot 2H_2O$)
5. urea + methionine hydroxy analogue
6. urea + sodium sulfate + methionine hydroxy analogue

For this experiment, it was desired to feed a finishing-type diet relatively high in concentrates which would result in rapid rates of gain. At the same time, a diet was preferred which was relatively low in protein requiring substantial amounts of supplemental protein to be added to the diet. Ground ear corn was selected since corn grain is lower in protein than most other feed grains. Furthermore, most common sources of roughage would provide more protein than the cob portion of ear corn.

Ground ear corn fed for the first three months of the experiment was field harvested at approximately 30% moisture. It was ground with a hammer mill and stored in an 18 ft. x 50 ft. concrete stave silo. The silo was later refilled with ear corn to which water was added to bring the moisture content up to approximately 20%. Ground ear corn from the field was ensiled with a medium to coarse degree of fineness whereas the reconstituted ear corn was processed to have a relatively fine degree of fineness. The cattle were fed 5 lb. of high moisture ground ear corn per head daily at the beginning of the experiment and this was increased at 1 lb. per head daily until a full feed was obtained. In addition to feeding ground ear corn, supplements were fed at 2 lb. per head daily to all treatment groups. The supplements were formulated to provide the dietary treatment variables. Chemical analyses of the ear corn and supplements and the ingredient composition of the protein supplements are presented in tables 1 and 2. All analyses were performed by A.O.A.C. methods (A.O.A.C., 1960).

TABLE 1. CHEMICAL COMPOSITION OF EAR CORN AND PROTEIN SUPPLEMENTS (MOISTURE-FREE BASIS)

| | Ground ear corn | Soybean control | Urea control | Urea + sodium sulfate | Urea + calcium sulfate | Urea + MHA | Urea + sodium sulfate + MHA |
|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % |
| Protein | 9.25 | 40.84 | 42.96 | 40.19 | 43.21 | 43.19 | 42.44 |
| Calcium | .012 | 4.79 | 4.00 | 3.24 | 4.68 | 3.78 | 3.46 |
| Phosphorus | .28 | .54 | .67 | .24 | .67 | .69 | .58 |
| Sulfur | .092 | .318 | .143 | .789 | .602 | .189 | .866 |
| Calculated nitrogen to sulfur ratio | 16.29 | 20.53 | 48.04 | 8.15 | 11.48 | 36.56 | 7.84 |

TABLE 2. INGREDIENT COMPOSITION OF SUPPLEMENTS FOR EXPERIMENT

| Ingredient | Type of supplement | | | | | |
|---|---|---|---|---|---|---|
| | Soybean control | Urea control | Urea + sodium sulfate | Urea + calcium sulfate | Urea + MHA | Urea + sodium sulfate + MHA |
| | % | % | % | % | % | % |
| Soybean meal | 85.53 | | | | | |
| Ground corn | | 72.93 | 70.73 | 71.53 | 72.60 | 70.40 |
| Urea (281%) | | 11.10 | 11.10 | 11.10 | 11.10 | 11.10 |
| Limestone | 8.80 | 8.30 | 8.30 | 6.70 | 8.30 | 8.30 |
| Trace mineral salt | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Dicalcium phosphate | | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Antibiotic premix[a] | .35 | .35 | .35 | .35 | .35 | .35 |
| Diethylstilbestrol premix[b] | .25 | .25 | .25 | .25 | .25 | .25 |
| Vitamin A premix[c] | .07 | .07 | .07 | .07 | .07 | .07 |
| Sodium sulfate | | | 2.20 | | | 2.20 |
| Calcium sulfate | | | | 3.00 | | |
| Methionine hydroxy analogue | | | | | .33 | .33 |

[a]Chlortetracycline at 35 mg per pound of supplement
[b]Diethylstilbestrol at 5 mg per pound of supplement
[c]10,000 I.U. vitamin A per pound of supplement

Urea supplements were formulated using corn grain, feed grade urea, calcium carbonate and dicalcium phosphate to be approximately equal to the soybean meal control supplement in protein, calcium and phosphorus content which were 37.6%, 3.6% and 0.58%, respectively. Urea was added at 11.1% by weight of the protein supplement. Inorganic sulfur, when added to urea supplements, was included to provide 1 part sulfur for each 10 parts nitrogen supplied from urea. This ratio of sulfur to nitrogen has been shown to best meet the animal's sulfur requirement in relation to nitrogen (Rozgoni, 1961; Moir et al., 1967). This is also the approximate ratio to which these elements exist in most commonly used feedstuffs (NRC, 1969). Sulfur sources were calcium sulfate (16.8% sulfur) and sodium sulfate (22.6% sulfur) and the methionine hydroxy analogue was added to provide 3 g per head daily. When calcium sulfate was used, the calcium level was adjusted to that of the other supplements by reducing the amount of calcium carbonate added. Trace mineral salt was added to all diets at a level of 5% by weight of the protein supplement while chlortetracycline, diethylstilbestrol and vitamin A were supplied to furnish 70 mg, 10 mg and 20,000 I.U. per head daily, respectively.

Feeding was once daily in outside paved pens without shelter. Feed was fed in fenceline bunks and water was provided from an automatic watering cup connected to a continuous circulating system. Cattle were weighed at 28-day intervals and these weights were used to calculate periodic gains and feed efficiency.

During the course of the experiment, two steers died of causes believed to be unrelated to the dietary treatments. In each case the steer was assigned a feed intake of the average per head for the pen up to the time of death and that amount was subtracted from the total consumption for the pen. This was done in order that feed consumption and feed efficiency could be calculated for the remaining steers.

Final shrunk weights were taken after feed and water were withheld for about 18 hours. Weight gains for the experiment were calculated on the basis of initial and final shrunk weights. On completion of an additional 4-week diethylstilbestrol-withdrawal study, carcass data were collected about 20 hours after slaughter. A government grader placed conformation grade, overall carcass grade and marbling score on each animal. The hot carcass weight was obtained and used in calculating dressing percent.

An analysis of variance was performed on the data using the Least-squares method and Dunnett's procedure was used to test significance between the means as outlined by Steel and Torrie (1960).

# RESULTS AND DISCUSSION

In this experiment, the primary objective was to determine the response from sulfur and methionine hydroxy analogue additions to high-urea supplements fed in ground ear corn diets. It was considered desireable to have a soybean meal supplement to serve as a positive control in evaluating performance obtained from urea. The urea control treatment was to be used as the basis for evaluating the responses obtained from the two sulfur compounds and methionine hydroxy analogue. Therefore, Dunnett's test was used for the test of significance in comparing means of the various treatments to the urea control.

Results of the feedlot performance are presented in table 3. The performance of steers fed a ground ear corn diet supplemented with 2 lb. daily of 37.6% protein supplements was quite good for all treatments with the average growth rate being 2.72 lb. per day.

All supplements were calculated to be isonitrogenous and approximately 80% of the nitrogen was furnished by urea in those which contained urea. This amount of urea, providing about 25% of the total dietary protein, presented no apparent palatability problems and weight gain for the urea control supplement was about equal to that of the soybean control. The slightly higher feed intake for steers fed the urea supplement with about the same rate of gain as for those fed soybean meal resulted in a slight increase in feed requirements ($P<.05$). This would indicate that urea as the primary supplemental protein to the ear corn diet did not affect weight gain but that it was utilized slightly less efficiently than soybean meal. The percentage of the

TABLE 3. SULFUR AND METHIONINE SUPPLEMENTATION WITH UREA FOR FEEDLOT CATTLE
(February 17 to September 29, 1971 - 224 days)

| Item | Type of supplement | | | | | |
|---|---|---|---|---|---|---|
| | Soybean control | Urea control | Urea + sodium sulfate | Urea + calcium sulfate | Urea + MHA | Urea + sodium sulfate + MHA |
| Number of steers | 16 | 15 | 15 | 16 | 16 | 16 |
| Initial shrunk wt., lb. | 484 | 486 | 480 | 490 | 487 | 484 |
| Final shrunk wt., lb. | 1092 | 1091 | 1099 | 1112 | 1089 | 1084 |
| Av. daily gain, lb. | 2.71 | 2.70 | 2.76 | 2.78 | 2.69 | 2.68 |
| Av. daily feed, lb. | | | | | | |
| Ground ear corn | 20.68 | 21.37 | 20.75 | 20.63 | 20.72 | 20.35 |
| Protein supplement | 1.99 | 1.99 | 1.99 | 1.99 | 1.99 | 1.99 |
| Total | 22.67 | 23.36 | 22.74 | 22.62 | 22.71 | 22.34 |
| Feed per 100 lb. gain, lb. | 835[a] | 865 | 823[a] | 814[b] | 844 | 834[a] |

[a]Significantly different (P<.05) from urea control
[b]Significantly different (P<.01) from urea control

total dietary protein from urea was less than the upper limit considered to be safe to feed and to be utilized efficiently. Since ground ear corn contains a lower level of protein than would be encountered in most diets commonly fed to finishing cattle, conditions requiring higher levels of supplementation with urea would appear unlikely with high-energy diets.

Highest rates of gain were obtained in this experiment when the urea-containing supplements were supplemented with the two sulfur compounds. These differences amounted to an average of only about 3% more when compared to the urea control group and were not statistically significant. There was essentially no difference in rate of gain for steers fed diets containing sodium sulfate or calcium sulfate.

Feed intake was lower when the sulfur compounds were added to the diets. The lower feed consumption with slightly higher rates of gain resulted in lower feed requirements for the sulfur-supplemented groups in comparison to the urea control. The improvement over the urea control was greater for calcium sulfate ($P<.01$) than for sodium sulfate ($P<.05$).

Some researchers have reported a beneficial effect from adding sulfur when urea composed a large part of the total protein in the diet (Jones and Haag, 1946; Brown et al., 1960). From chemical analyses presented in table 1, ground ear corn had a calculated nitrogen to sulfur ratio of 16.29 to 1. The ratio in the urea control supplement was 48.04 to 1. These values would indicate a sulfur deficiency according to Loosli (1952), Rozgoni (1961) and Moir et al. (1967).

While rate of gain for the urea control group was about equal to that made by the steers fed soybean meal, they did have higher feed requirements. The small increase in rate of gain with a lower feed intake observed with the sulfur supplements might indicate an improvement in feed utilization when sulfur was added to the urea-containing diets. The nitrogen to sulfur ratio in diets with added sulfur was 15.72 to 1. In comparison to the urea control, calculated to have a nitrogen to sulfur ratio of 19.08 to 1, the added sulfur was instrumental in providing a more optimum ratio.

Calcium sulfate appears to have been used to a very limited extent as a sulfur supplement for cattle. Results of this experiment would indicate it to be as efficient as sodium sulfate as a sulfur supplement when urea is used as the major source of supplemental protein. Calcium sulfate is a relatively cheap source of sulfur in comparison to sodium sulfate. In addition, the contribution it makes in calcium would be an added value in finishing type diets which are generally low in this element in relation to requirements for cattle.

When the methionine hydroxy analogue was added to provide 3 g per head daily with the urea supplement, rate of gain was about the same as for steers fed the urea control diet. Feed intake was reduced by the methionine addition to the diet, and there was a slight but nonsignificant reduction in feed requirement. These results would indicate no beneficial effect from methionine hydroxy analogue supplementation to a diet with urea used as the major source of supplemental protein. This is in contrast to an apparent improvement from either the calcium sulfate or sodium sulfate supplements. Diets with added sulfur contained a nitrogen to sulfur ratio of 15.72 to 1 in comparison to 18.07 to 1 for those with only methionine addition.

Methionine hydroxy analogue supplementation in combination with sodium sulfate also did not result in any improvement in weight gains in comparison to the urea control. This treatment resulted in the lowest feed intake in the experiment. Feed requirements were lower ($P<.05$) than for the urea control; they were slightly less than for the methionine without sodium sulfate and slightly higher than for sodium sulfate without added methionine.

These results also indicate no beneficial effect from methionine supplementation in comparison to performance of steers fed the urea control diet. It also appears that the methionine hydroxy analogue in addition to sulfur supplementation had a slight depressing effect on performance of the cattle. A definite "methionine" aroma was detectable from supplements containing the methionine hydroxy analogue in this experiment. This may have been a contributing factor which resulted in the depressing effect observed on daily feed intake for those diets with methionine analogue-containing supplements.

Burroughs et al. (1969, 1970) found a marked response from feeding 3 g of methionine hydroxy analogue daily to heifers for 151 days and to steers for the initial 72-day period of a 133-day feeding period where the diet was rolled corn and cobs supplemented with urea.

Heifers gained 13% faster than controls while steers showed a 7% improvement in rate of gain. These results were in contrast to those of Gossett et al. (1962), Beeson et al. (1970) and Hale et al. (1970). Gossett et al. (1962), in feeding 5 or 10 g of methionine analogue daily in a high-urea supplement with ground corn and corn silage, found that the 5-g level was of no benefit and the 10-g level significantly depressed rate of gain. Results by Hale et al. (1970) showed similar findings when experimenting with 22 g of methionine hydroxy analogue per head daily in milo diets supplemented with urea. No response in rate of gain was observed at any time during the author's experiment from feeding 3 g of methionine hydroxy analogue per head daily in an ear corn diet. Under similar dietary conditions, Beeson et al. (1970) found cattle gained 7% less than controls.

Data pertaining to the various carcass characteristics are presented in table 4. All differences were small and nonsignificant and did not appear to be affected by dietary treatments. This might be expected due to the small differences in rate of gain and similar final weights at which the cattle were marketed.

TABLE 4. CARCASS CHARACTERISTICS OF CATTLE FED SULFUR AND METHIONINE SUPPLEMENTS WITH UREA

| Item | Type of supplement | | | | | |
|---|---|---|---|---|---|---|
| | Soybean control | Urea control | Urea + sodium sulfate | Urea + calcium sulfate | Urea + MHA | Urea + sodium sulfate + MHA |
| Carcass wt., lb. | 738 | 742 | 728 | 738 | 731 | 730 |
| Conformation[a] | 21.8 | 21.3 | 21.6 | 21.5 | 21.5 | 21.5 |
| Marbling[b] | 5.3 | 5.7 | 5.1 | 5.0 | 5.4 | 5.3 |
| Carcass grade[a] | 18.5 | 19.1 | 18.2 | 18.2 | 18.6 | 18.3 |
| Dressing percent | 63.0 | 63.0 | 61.7 | 62.2 | 62.6 | 63.0 |

[a]Choice = 20; Good = 17. Graded to one-third of a grade.
[b]Small = 5; modest = 6.

# SUMMARY

Ninety-six Hereford steer calves weighing approximately 500 lb. were used in a 224-day experiment and were full-fed ground ear corn with 2 lb. daily of a 37.6% protein supplement. Experimental dietary treatments consisted of a soybean meal control, urea control, urea + sodium sulfate, urea + calcium sulfate, urea + methionine hydroxy analogue and urea + sodium sulfate + methionine hydroxy analogue. The experiment was designed to study the effects of added sulfur and methionine to high-urea supplements fed in ground ear corn diets for growing and finishing beef cattle.

Urea-containing supplements which provided about 25% of the total dietary protein were calculated to be isonitrogenous with the soybean meal control supplement. Performance data for all dietary treatments was good with the urea control group being about equal in rate of gain to that of the soybean meal group. Feed intake for the urea control group was greater, indicating that the urea diet was being utilized less efficiently than the diet with soybean meal.

Additions of inorganic sulfur in the form of sodium sulfate or calcium sulfate, supplemented at a rate of 1 part sulfur to each 10 parts nitrogen from urea, resulted in higher but nonsignificant weight gains. The improvement for both sources of sulfur over the urea control supplement was about 3%. Sulfur additions significantly improved feed efficiency. Improvements were slightly greater for calcium sulfate ($P<.01$) than for sodium sulfate ($P<.05$). There was

essentially no difference in feedlot performance between the two added sources of sulfur.

Essentially no difference in rate of gain was obtained when the methionine hydroxy analogue was added to the urea control supplement. Feed intake was lower when methionine analogue was added to the supplement; however, these differences compared very closely with those obtained when inorganic sulfur was added to the supplement. Methionine hydroxy analogue supplementation in combination with sodium sulfate also did not result in any improvement in weight gain in comparison to the urea control. This treatment resulted in the lowest feed intake in the experiment.

Carcass characteristics did not appear to be influenced by experimental treatments under the conditions of this experiment. This could be explained from the small differences observed in average daily gain and similar weights at which cattle were marketed.

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